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Isolation and molecular characterization of *Toxoplasma gondii* from chickens and ducks from Egypt

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Abstract

The prevalence of *Toxoplasma gondii* in free range chickens is a good indicator of the prevalence of *T. gondii* oocysts in the environment because chickens feed from the ground. In the present study, prevalence of *T. gondii* in 121 free range chickens (*Gallus domesticus*) and 19 ducks (*Anas* sp.) from a rural area surrounding Giza, Egypt was assessed. Blood, heart, and brain from each animal were examined for *T. gondii* infection. Antibodies to *T. gondii*, assayed with the modified agglutination test (MAT), were found in 49 (40.4%) chickens in titers of 1:5 in 11, 1:10 in four, 1:20 in four, 1:40 in eight, 1:80 in 10, and 1:160 or more in 12 chickens. Antibodies were found in three ducks each with a titer of 1:80. Hearts and brains of seropositive (MAT \geq 1:5) chickens and ducks were bioassayed in mice. Additionally, hearts and brains of seronegative (MAT < 1:5) animals were bioassayed in *T. gondii*-free cats. *T. gondii* was isolated from 19 of 49 seropositive chickens (one with a titer of 1:5, two with a titer of 1:20, one with a titer of 1:40, five with a titer of 1:80, three with a titer of 1:160, and seven with a titer of \geq 1:360). One cat fed tissues pooled from 15 seronegative chickens shed *T. gondii* oocysts, while two cats fed tissues of 34 seronegative chickens did not shed oocysts. *T. gondii* was isolated from one of the seropositive ducks by bioassay in mice. The two cats fed tissues from 16 seronegative ducks did not shed oocysts. Genotyping of 20 chicken isolates of *T. gondii* using the SAG 2 locus indicated that 17 isolates were type III and three were type II. The duck isolate of *T. gondii* was type III. The mice inoculated with tissue stages of all 21 isolates of *T. gondii* from chickens and ducks remained asymptomatic, indicating that phenotypically they

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were not type I because type I strains are lethal for mice. Infections with mixed genotypes were not found.

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1. Introduction

Toxoplasma gondii infections are widely prevalent in human beings and animals world-wide (Dubey and Beattie, 1988). Humans become infected postnatally mainly by ingesting tissue cysts from undercooked meat or from the food or drink contaminated with oocysts shed in cat feces. However, only a small percentage of exposed adult humans or animals develop clinical signs. It is unknown whether the severity of *T. gondii* infections is due to the parasite strain, quantum of infection, host immunity, or other factors. Overall, there is low genetic diversity among *T. gondii* isolates so far examined. *T. gondii* strains have been classified into three genetic types (I, II, III) (Howe and Sibley, 1995; Howe et al., 1997).

It has been suggested that type I isolates or recombinants of types I and III are more likely to result in clinical toxoplasmosis (Howe et al., 1997; Grigg et al., 2001a; Fuentes et al., 2001; Aspinall et al., 2003), but genetic characterization has been limited essentially to patients ill with toxoplasmosis. Contrary to humans, most isolates of *T. gondii* obtained from animals and genetically typed were type II or type III, irrespective of the clinical status of the animal (Howe and Sibley, 1995; Mondragon et al., 1998; Owen and Trees, 1999; Jungersen et al., 2002). Recently, 17 of 25 isolates of *T. gondii* obtained from asymptomatic-free range chickens from rural areas surrounding São Paulo, Brazil were classified as type I (Dubey et al., 2002). Because chickens become infected mostly by feeding from the ground contaminated with oocysts, prevalence of *T. gondii* in chickens is a good indicator of prevalence of *T. gondii* in their environment (Ruiz and Frenkel, 1980).

Little is known of the biological and molecular characteristics of isolates of *T. gondii* from the Middle East and Africa. The purpose of this study was to isolate *T. gondii* from poultry from Egypt and to genetically characterize them.

2. Materials and methods

2.1. Naturally infected chickens and ducks

Three batches of free range chickens and ducks from 11 villages in rural areas in Egypt were purchased, bled, killed, and necropsied (Tables 1 and 2). The first batch was from four villages in Menofia Governorate, 60 km north of Cairo and the birds were killed on 18 April 2002. The second batch was from three villages in El-Bihera Governorate, 200 km northwest of Cairo and these birds were killed 2 June 2002. The third batch was from four villages in El-Bihera Governorate, 180 km from Cairo. The houses in a village are very

Table 1
Prevalence of *T. gondii* in chickens from Giza, Egypt

Batch No.	No. of chickens	No. of chickens with <i>T. gondii</i> infection		
		Antibodies (titers)	Bioassay	
			In mice	In cats
1	30	11 (1:5 in three, 1:20 in two, 1:40 in two, 1:80 in one, 1:160 in one, $\geq 1:320$ in two)	7	0/19 ^a
2	41	19 (1:5 in four, 1:10 in two, 1:40 in two, 1:80 in six, 1:160 in one, $\geq 1:320$ in four)	4	ND ^b
3	50	19 (1:5 in four, 1:10 in two, 1:20 in two, 1:40 in four, 1:80 in three, 1:160 in two, $\geq 1:320$ in two)	8	1/15, 0/15

^a No. of cats that shed oocysts/no. of chicken tissues fed.

^b ND: not done because tissues were autolyzed.

close to each other, located within approximately 0.5 km diameter. The villages are about 2 km apart from each other.

Heart, head and serum from each animal were transported by air to the USDA's laboratory in Beltsville, Maryland for *T. gondii* examination. The samples were received in three batches (April, June, August, 2002) and 4–5 days elapsed between killing and bioassay for *T. gondii*.

Table 2
Isolation of *T. gondii* from tissues of chickens from Egypt

Batch No.	Chicken No.	Village designation	MAT titer of infected chicken	No. of mice positive/no. inoculated	Genotype ^a
1	7 (2)	A	20	2/5	II
	9 (3)	A	≥ 320	5/5	III
	10 (4)	B	40	5/5	III
	22 (8)	C	160	5/5	III
	25 (9)	C	≥ 320	5/5	III
	28 (10)	C	80	2/5	III
	30 (11)	D	20	2/5	III
2	8 (13)	E	≥ 320	2/2	III
	20 (20)	F	160	2/2	III
	28 (22)	F	80	1/2	III
	40 (29)	G	80	1/2	III
3	18 (32)	H	≥ 320	1/5	III
	25 (35)	I	80	5/5	III
	26 (36)	I	≥ 320	5/5	III
	29 (38)	I	160	5/5	II
	37 (42)	J	80	3/5	III
	45 (45)	K	≥ 320	4/5	III
	48 (47)	K	≥ 320	5/5	III
	49 (48)	K	5	3/5	II

^a The 20th isolate obtained by cat bioassay was type III.

2.2. Serologic examination

Sera from chickens were diluted twofold starting at 1:5 dilution and assayed for *T. gondii* antibodies with the modified agglutination test (MAT) as described (Dubey and Desmonts, 1987).

2.3. Bioassay of poultry tissues in cats

Hearts and brains from 49 seronegative (MAT < 1:5) chickens (Table 1) and 16 ducks (from batches 1 and 3) were pooled in batches and fed to five cats. Feces of cats were examined for *T. gondii* oocyst shedding as previously described (Dubey, 1995). Oocysts were suspended in 2% H₂SO₄, allowed to sporulate at room temperature, and bioassayed in mice (Dubey and Beattie, 1988).

2.4. Bioassay of poultry tissues in mice

The procedures were identical to those described by Dubey et al. (2002). Brains and hearts of seropositive (MAT ≥ 1:5) chickens and ducks were bioassayed individually in mice after digestion in pepsin (Dubey, 1998). For this, brain and heart of each animal were pooled, homogenized in five volumes (w/v) of aqueous 0.85% NaCl (saline), mixed with five volumes of acidic pepsin and the mixture incubated in a shaker water bath for 1 h at 37 °C. The digest was centrifuged, neutralized, mixed with antibiotics, and the homogenate was inoculated subcutaneously (s.c.) into five (batches 1, 3) or two (batch 2) mice. The mice used were Swiss Webster albino females obtained from Taconic Farms, Germantown, New York. Tissue imprints of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 45 post-inoculation (p.i.), and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies by MAT. Mice were killed 50 days p.i. and their brains were examined microscopically for tissue cysts, and a portion of the brain was frozen for DNA extraction. Mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were demonstrable in their tissues.

2.5. Genetic characterization

T. gondii DNA was extracted from infected mouse tissues as described by Lehmann et al. (2000). PCR–restriction fragment length polymorphism (RFLP) genotypes of SAG2 locus was used to determine the genetic type (Howe et al., 1997).

3. Results

Antibodies to *T. gondii* (≥ 1:5) were found in 49 of 121 (40.4%) chickens, and three of 19 (15.7%) ducks. The MAT titers of chickens were 1:5 in 11 chickens, 1:10 in four chickens, 1:20 in four chickens, 1:40 in eight chickens, 1:80 in 10 chickens, 1:160 in four chickens, and 1:320 or more in eight chickens (Table 1). The MAT titer of the three positive ducks was 1:80.

T. gondii was isolated from tissues of 19 seropositive chickens from 11 villages (Table 2). The isolation rate varied in three batches of chickens. *T. gondii* was isolated from seven of 11 (63.6%), four of 19 (21.05%), and eight of 19 (42.10%) seropositive chickens in batches 1, 2, and 3, respectively (Table 2). *T. gondii* was isolated from one of the 11 (9.1%) chickens with a titer of 1:5, two of the four (50%) with titer of 1:20, one of eight (12.5%) with titer of 1:40, five of 10 (50%) with titer of 1:80, and 10 of 12 (83.3%) with titers of 1:160 or more. Overall, the probability of isolating *T. gondii* in mice increased with the antibody titer. All mice inoculated with *T. gondii*-infected chicken or duck tissues remained asymptomatic and tissue cysts were found in their brains when euthanized 6 weeks later.

One cat fed tissues of 15 seronegative chickens shed *T. gondii* oocysts (Table 1). The oocysts were lethal to mice by the oral route but mice inoculated with tachyzoites of this isolate did not die and tissue cysts were found in their brains when euthanized 2 months later. The other two cats fed tissues of 34 seronegative chickens did not shed oocysts.

T. gondii was isolated from one of the three seropositive ducks. The two cats fed with tissues of the 16 seronegative ducks did not shed oocysts.

Of the 19 isolates of *T. gondii* from chickens obtained by mouse bioassay, three were type II and 16 were type III (Table 2). The 20th chicken isolate obtained by cat bioassay, and the duck isolate were both type III. Infections with mixed genotypes were not found.

4. Discussion

T. gondii was isolated from seven of nine seropositive chickens in batch 1, four of 18 in batch 2, and eight of 19 in batch 3. This low *T. gondii* isolation rate from batch 2 was probably because tissues of chickens were badly autolyzed due to inappropriate storage temperature; these tissues were not bioassayed in cats.

In a previous survey of poultry from Giza, Egypt, *T. gondii* antibodies (MAT \geq 1:25) were found in 47% of 108 chickens (El-Massey et al., 2000). In the present study, antibodies were found in 40.4% of 121 chickens (MAT \geq 1:5) of which only 28% had titers \geq 1:20. In the present study, sera were assayed starting at a lower dilution (1:5) compared with 1:25 dilution used previously (El-Massey et al., 2000) because occasionally *T. gondii* has been isolated from chickens with antibodies lower than 1:25 (Dubey et al., 2002). *T. gondii* was isolated from three chickens with a titer of $<$ 1:25; from one chicken with a titer of 1:5, two chickens with a titer of 1:20. Additionally, *T. gondii* was isolated from the feces of a cat fed tissues pooled from 15 seronegative ($<$ 1:5) chickens. The lack of isolation of *T. gondii* from the 34 seronegative chickens and none of the 16 seronegative ducks suggests that the incidence of false negative results with MAT is low. Among all serologic assays tested, the MAT was the most sensitive and specific test for detecting latent *T. gondii* infection in chickens (Dubey et al., 1993). The cat is a very sensitive indicator of *T. gondii* infection because cats fed even a few *T. gondii* bradyzoites can shed millions of oocysts (Dubey, 2001). Therefore, cats were fed tissues of chickens considered to be seronegative to validate the serology.

In the present study, *T. gondii* was isolated from tissues of one of 19 ducks. To our knowledge, this is the first confirmed report of isolation of *T. gondii* from tissues of an asymptomatic domestic duck. Boehringer et al. (1962) described an outbreak of toxoplasmosis in

a flock of hundred 2–3-week-old ducks in which 50% of the ducks died within 5 days. *T. gondii* was found in mice inoculated with tissues of ducks.

T. gondii isolates differ markedly in their virulence to outbred mice. Type I isolates are lethal to mice, irrespective of the dose and virulence is genetically controlled (Howe et al., 1996; Grigg et al., 2001b; Su et al., 2002). As all mice inoculated with infected chicken or duck tissues survived, *T. gondii* isolates in this study were not type I phenotypically. Genetically, 85% of the chicken isolates from Egypt were type III, while type I isolates were not recovered. These findings are different from the *T. gondii* isolates from free range chickens from Brazil, which were predominantly type I (Dubey et al., 2002), and other *T. gondii* isolates from mammals from North America and Europe, which were predominantly type II (Mondragon et al., 1998; Owen and Trees, 1999; Jungersen et al., 2002).

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